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The neutralizing capacity of antisera can be quantitated by utilizing an assay which will detect single round of HIV-1 entry. Recombinant HIV-1 lacking *env* and possessing a CAT reporter gene are complemented in *trans* with various envelope glycoproteins, allowing infection of CD4-positive T-

5 cells. This assay can be used to determine the titer of homologous neutralization as well as the breadth of the neutralizing response by assessing the capacity of the antisera to cross-neutralize viruses with heterologous laboratory-adapted and primary envelope glycoproteins.

These selectively deglycosylated polypeptides should elicit antibodies

10 capable of neutralizing a broad range of laboratory-adapted strains better than wild-type glycoproteins. In generating an immune response, the presence of such a range of antibodies will have a valuable role by significantly diminishing the acute viremia phase observed in most individuals soon after infection with HIV-1. Those glycoproteins containing

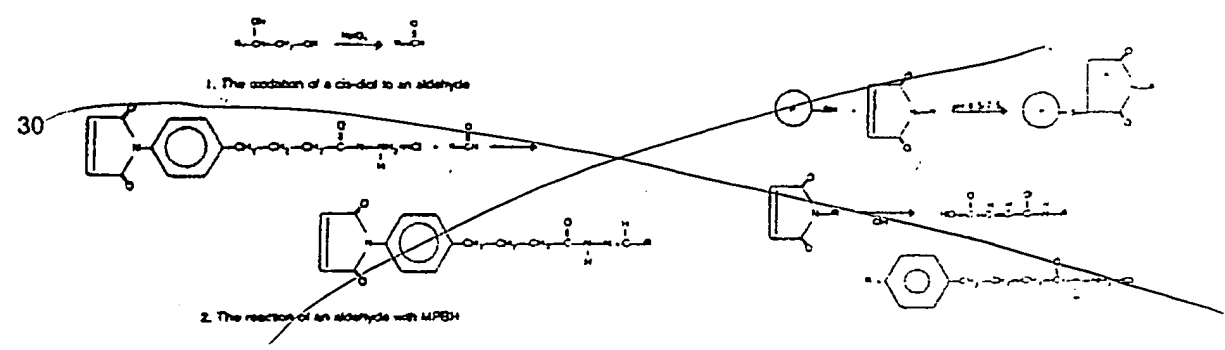
15 multiple glycosylation site mutations as opposed to the wild-type are preferred.

To derivatize the sugars distal from gp120 receptor binding regions, gp120 sugar residues containing vicinal hydroxyls will be oxidized to aldehydes using sodium meta-periodate (NaIO_4 , Reaction 1). These

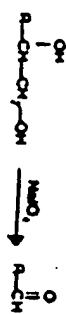
20 aldehydes can then be reacted with the hydrazide group on the heterobifunctional cross-linker MPBH (Pierce) to form hydrazones (Reaction 2).

The MBPH also contains a reactive melamide group, which is selective for sulfhydryls when the pH of the mixture is kept between 6.5 and

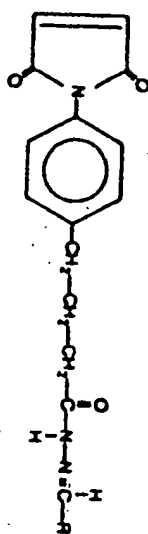
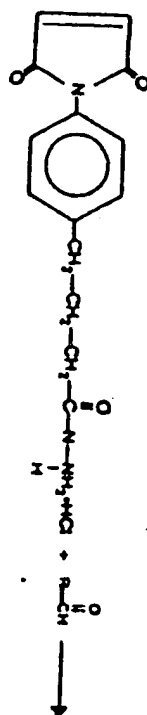
25 7.5 (Reaction 3). In *Drosophila* cells, we will produce recombinant murine C3d that has been genetically altered to remove its reactive group



09/04/99



1. The oxidation of a cis-diol to an aldehyde



2. The reaction of an aldehyde with MPBH

